Rejection under 35 U.S.C. § 102(e)

The rejection of claims 65-72, 74, 77, and 78 under § 102(e) as being anticipated by Sherwin et al. (U.S. Patent No. 6,015,708) has been maintained, with the Examiner stating that Sherwin, at column 3, fourth full paragraph, teaches the expression of a gene product of interest in cells that have been modified using homologous recombination. Applicants respectfully disagree with the Examiner's characterization of this paragraph, which is reproduced as follows:

> After amplification, by employing the amplifiable gene, the transformed hosts are then screened for production of the target protein and stability and derivative cell lines are selected for desired levels of production, which cells may be expanded and used for production of the desired protein in culture. (Emphasis added.)

This paragraph thus states that expression of a target protein is detected in transformed host cells, but this term is not defined in the paragraph. Thus, to determine what is meant by "transformed hosts," it is necessary to consider the preceding paragraph in which this term is defined. The second full paragraph of column 3 reads as follows:

> The YAC library is maintained and propagated in a yeast host and homologous recombination is then employed for integrating a DNA targeting construct, usually comprising an amplifiable gene for integration into a target region comprising the target gene, which target gene encodes the protein of interest, while also allowing for, in the same or separate step, manipulation of the transcriptional system and/or the coding region. The modified yeast cells may then be analyzed and sequences providing for the desired modifications identified. The amplifiable region may then, as appropriate, be transformed into the expression host and the amplifiable region amplified. (Emphasis added.)

From this paragraph it is clear that what is meant by "transformed hosts" in the fourth full paragraph of column 3 is expression hosts that have been transformed with sequences that have been manipulated by homologous recombination in a prior, separate cell (i.e., the "yeast host"). Homologous recombination is carried out to introduce a construct including an amplifiable gene into a yeast host, and the amplifiable region is

then transformed into an expression host. Thus, the transformed hosts of paragraph 4 of column 3, in which expression of a gene product of interest is detected, are not the same cells that have been modified by homologous recombination. In the event that the Examiner does not agree with this analysis, applicants respectfully request that he specifically explain how these paragraphs could be interpreted any differently than as is set forth above.

The Examiner also states that Sherwin teaches a two-part invention, with the first part being gene targeting followed by expression analysis to test for success, and the second part involving transfer of modified DNA to a second cell to improve further expression. Applicants respectfully disagree.

MUST LOOK AT '069 APPL. TEACHINGS

As is discussed above, Sherwin does not teach detection of expression of a gene product of interest in cells that have been modified by homologous recombination. This certainly is not taught at column 3, paragraph 4, for the reasons discussed above. It is also not taught elsewhere in the Sherwin patent. For example, in passages where Sherwin specifically discusses how modifications by homologous recombination are characterized, it is stated that this is done by detection of expression of a marker gene (column 5, lines 35-43), restriction analysis (column 5, lines 44-50; column 8, lines 33-39), sequencing (column 8, lines 33-39), hybridization (column 8, lines 33-39), or PCR (column 8, lines 33-39). In none of these passages, which are specifically directed to describing methods for characterizing homologous recombination-induced integration events, is detection of expression of a gene product of interest even mentioned.

This is also true of the experimental examples provided in the Sherwin patent. These examples describe (i) targeting of the FSH-β locus in yeast artificial chromosomes

(YAC's) in yeast cells, followed by transfer of the targeted FSH-β locus into CHO cells for expression of FSH-β (column 9, line 25 through column 14, line 6); (ii) targeting of the G-CSF locus in YAC's, followed by transfer of the targeted G-CSF locus into CHO cells for expression of G-CSF (column 14, line 7 through column 16, line 48); and (iii) targeting of the erythropoietin locus in human 293 embryonal kidney cells, followed by transfer of the targeted erythropoietin locus into CHO cells for expression of erythropoietin (column 16, line 49 through column 20, line 8). In the first two of these three examples, the homologous recombination event was detected by Southern blot analysis of restriction enzyme-digested DNA, not by analysis of expression of a gene product of interest in the cells in which homologous recombination took place (column 12, lines 64-67 and column 15, lines 48-51). Similarly, in the third example, the homologous recombination event was detected by PCR analysis (column 18, lines 7-65), not by detection of expression of a gene product of interest in the cells in which homologous recombination took place.

Thus, Sherwin nowhere teaches detection of expression of a gene product of interest in cells in which targeting by homologous recombination has taken place. This is not done in the paragraph noted by the Examiner (column 3, paragraph 4), not in the sections that specifically discuss how homologous recombination events are characterized in the methods of Sherwin (see above), and not in the experimental section. The rejection should thus not be maintained on this basis.

The Examiner has also maintained this rejection, stating that the Summary of the Invention of the Sherwin patent indicates that transfer of the DNA to the second cell is optional, because it states that the DNA "may then be transferred to a secondary host."

This passage does not mean that expression can be obtained in the absence of transfer of the modified sequence to a secondary host, but rather that if the option of obtaining expression is desired, then the DNA should be transferred. Interpreting this passage in any other manner is inconsistent with the teachings of the Sherwin patent as a whole. As is discussed at length above, nowhere in the Sherwin patent is there any teaching of expression of a gene product of interest in cells in which homologous recombination takes place. Applicants respectfully request that the rejection under § 102(e) over the Sherwin patent be withdrawn.

Rejection under 35 U.S.C. § 103(a)

The rejection of claims 65, 72, and 73 under § 103(a) for obviousness over Sherwin (U.S. Patent No. 6,015,708), in view of Capecchi (Science 244:1288-1292, 1989) has been maintained for the reasons discussed above with respect to the rejection under § 102(e) over the Sherwin patent. Applicants respectfully request that this rejection be withdrawn.

In the final Office Action, the Examiner stated that Sherwin differs from the claimed invention in not teaching the use of negative and positive selection markers, and that the use of such markers in the methods of Sherwin would have been obvious based on the teachings of Capecchi. In particular, the Examiner stated that Capecchi teaches the use of such markers, and that motivation to use them would have come from Capecchi's teaching that the use of these markers can lead to enrichment for desired cells.

As is discussed above and in applicants' previous replies, the teachings of Sherwin differ substantially from applicants' claimed invention. Sherwin teaches homologous recombination in a first cell, followed by transfer into a second, immortalized cell to achieve expression of a gene

product of interest. Sherwin does not teach expression of the gene product of interest in the cell in which homologous recombination takes place. In contrast, the present claims relate to the use of homologous recombination to activate expression of a gene product of interest in primary or secondary cells, in the absence of further gene transfer. Capecchi's teaching of positive and negative selection markers does not provide what Sherwin lacks in supporting a rejection of the present claims for obviousness. Applicants thus respectfully request that this rejection be withdrawn.

CONCLUSION

Applicants submit that the claims are in condition for allowance, and such action is respectfully requested. In addition, applicants request that the Examiner please contact the undersigned before issuing a further Office Action in this case, unless the further Office Action is a Notice of Allowance.

Although no charges are believed to be due, if there are any charges or any credits, please apply them to Deposit Account No. 03-2095.

Respectfully submitted,

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